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A Lipid-Peptide Microbicide Inactivates Herpes Simplex Virus

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A microbicide combining the lipid-ether 1-0-octyl-sn-glycerol (OG; 3 mM) and peptide D2A21 (9 μM) reduced herpes simplex virus type 1 (HSV-1) and HSV-2 titers by at least 1,000-fold, more than the sum of the inactivations produced by OG and D2A21 alone. OG plus D2A21 reduced HSV-1 and HSV-2 titers by ≥1,000-fold in ≤10 and ≤20 min, respectively, whereas OG and D2A21 used alone produced almost no virus inactivation during these times.

In the absence of a fully effective herpes simplex virus (HSV) vaccine, combination topical microbicides represent an important strategy for reducing HSV transmission (3, 6, 11, 12, 7). A combination microbicide will inactivate HSV prior to the initial infection of susceptible cells in the genital tract.

Studies in our laboratory have shown that the lipid ether 1-0-octyl-sn-glycerol (OG; Deva Biotech, Hatboro, Pa.) inactivates HSV and chlamydiae (1, 4, 2), but in the presence of 10% fetal bovine serum (FBS), viral inactivation took at least 1 h and required lipid concentrations of 10 to 15 mM. The present study was designed to determine whether combining OG with an antimicrobial peptide (14) would decrease the time required for HSV inactivation and reduce the concentration of antimicrobial lipid needed for viral killing. Preliminary studies in our laboratory (data not shown) indicated that the synthetic peptide D2A21 (Demegen, Pittsburgh, Pa.) had stronger anti-HSV activity than the magainins, defensins (13, 14), and lentivirus lytic peptides (9) tested. However, D2A21 concentrations of 30 and 60 μM only reduced the titer of HSV type 2 (HSV-2) by 25- and 200-fold, respectively, in 3 h.

HSV-1 and HSV-2 (American Type Culture Collection, Manassas, Va.) were grown in Vero and CV-1 cells, respectively, and assayed as previously described (10, 8). OG at a concentration of 3 mM was combined with concentrations of D2A21 ranging from 3 to 9 μM over a period of 1 h at 37°C in the presence of 1% FBS and examined for activity against HSV-1 and HSV-2 (Fig. 1). The effect of human serum on the anti-HSV activity of OG and D2A21 was the same as that of FBS (results not shown), but human serum was not used in these experiments because immune system-related elements present in most human serum will inactivate HSV and compromise the ability to monitor the direct effects of OG and D2A21. When OG was used alone against HSV-1 (Fig. 1A) it had a minimal effect on the viral titer after 10 min of incubation but reduced the titer of HSV-1 by 100-fold in 20 min and by 1,000-fold in 60 min. Nine micromolar D2A21 by itself did not inactivate HSV-1 after 60 min. When OG was combined with 3 μM D2A21, the HSV-1 titer was reduced by 100-fold at the zero time point (the time it took to dilute the incubation mixture for assay) and by 10,000-fold within 10 min, and no HSV-1 infectivity was detected at 30 min. Raising the D2A21 concentration to ≥6 μM in the presence of 3 mM OG completely inactivated HSV-1 in ≤10 min. OG by itself was less effective against HSV-2 (Fig. 1B) than against HSV-1, only reducing the HSV-2 titer by 10-fold after 60 min. As with HSV-1, 9 μM D2A21 alone had almost no effect on the HSV-2 titer. When OG plus 3 μM D2A21 was incubated with HSV-2, it took 60 min to reduce the HSV-2 titer by 100-fold; however, raising the D2A21 concentration to 6 μM produced a 100-fold reduction of HSV-2 infectivity in 10 to 20 min and an ~10,000-fold reduction in 40 min. HSV-2 was completely inactivated by 40 min with OG combined with 9 μM D2A21. The combination of OG and 9 μM D2A21 decreased the HSV-2 titer by 100-fold at the zero time point, whereas each compound when used by itself had no effect on viral activity. This effect was also found with HSV-1 (Fig. 1A) at the zero time point where OG and 9 μM D2A21 used individually did not reduce the HSV-1 titer but when combined reduced viral infectivity by 1,000-fold. These experiments showed that combining OG and D2A21 has a synergistic effect not only on the peptide concentration required but also on the time required for HSV inactivation. With viruses such as HSV (and human immunodeficiency virus) (3), which establish permanent infections, decreasing the viral titer as quickly as possible to prevent the initial infection of susceptible cells is one of the keys to reducing viral prevalence (5).

Experiments were then performed to determine what effect increasing the serum concentration to 10%, which increases the concentration of compounds that bind nonspecifically to lipids and peptides, has on the effectiveness of the antiviral mixtures (Fig. 2). The time required for HSV-1 inactivation was increased in the presence of 10% serum (Fig. 2A) compared to 1% serum (Fig. 1A). OG by itself only decreased the HSV-1 titer by 10- to 30-fold after 60 min. At the zero time point no HSV-1 killing was found when OG and 9 μM D2A21 were used together with 10% serum. Significant HSV-1 inactivation took 10 min of incubation, at which point OG combined with 3, 6, and 9 μM D2A21 reduced HSV-1 titers by 100-, ≥1,000-, and ≥10,000-fold, respectively. Nine micromolar D2A21 combined with 3 mM OG completely inactivated

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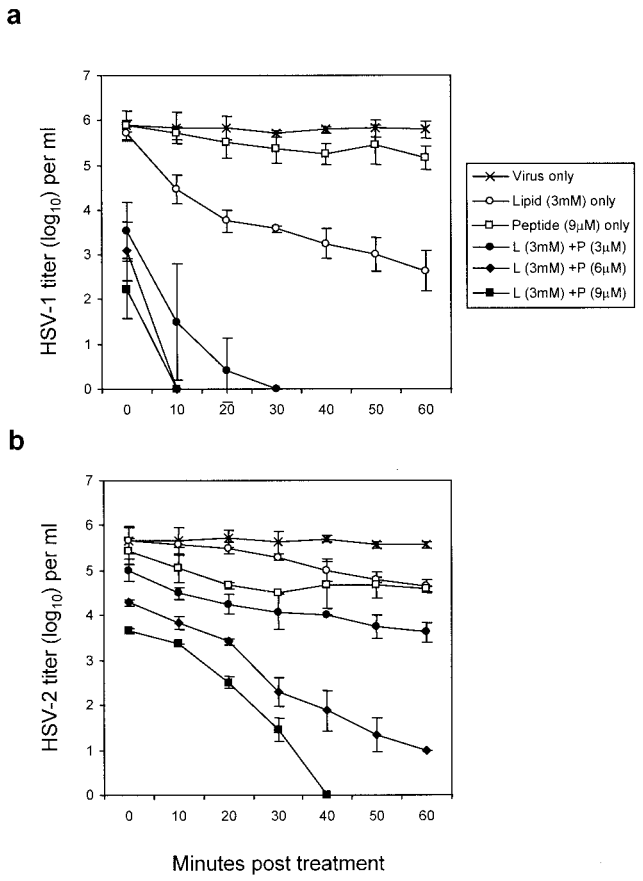

FIG. 1. Inactivation of HSV-1 (A) and HSV-2 (B) by mixtures of OG (L) and D2A21 (P) in the presence of 1% FBS. Each point is the mean ± the standard deviation for three separate experiments. The zero time point is the time it took to dilute the incubation mixture for assay.

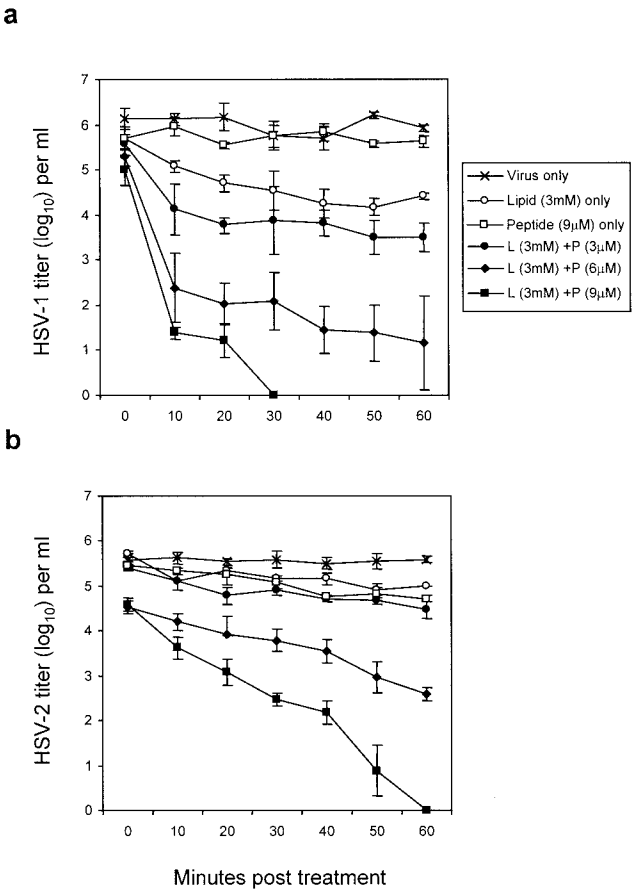

FIG. 2. Inactivation of HSV-1 (A) and HSV-2 (B) by mixtures of OG (L) and D2A21 (P) in the presence of 10% FBS. Each point is the mean ± the standard deviation for three separate experiments.

HSV-1 by 30 min. The results obtained with 10% serum and HSV-2 (Fig. 2B) show that the time required for complete HSV-2 inactivation was lengthened to 60 min from 40 min compared to studies with HSV-2 in the presence of 1% serum (Fig. 1B). However, HSV-2 infectivity was reduced by 100-fold at 10 min, 1,000-fold at 30 min, and almost 100,000-fold at 50 min in the presence of 10% serum, 3 mM OG, and 9 μM D2A21.

In the presence of both 1 and 10% FBS, a greater time of exposure is required to inactivate HSV-2 than HSV-1 with all of the lipid-peptide mixtures tested. This may result from the somewhat greater sensitivity of HSV-1 to OG, which can be seen by comparing Fig. 1A and B. Since similar starting virus titers were used, this difference in sensitivity to lipid between these two similar viruses could result from their being grown in two different cell types (Vero and CV-1 cells) or possibly from differences in the glycoproteins in their envelopes. Our results demonstrate that combining an antiviral lipid with an antiviral peptide, thus targeting the HSV envelope simultaneously by two separate mechanisms, produces a microbicide that more effectively inactivates HSV than when each of the active compounds is used individually. This combination microbicide can decrease the time required to reduce HSV-1 and HSV-2 infectivity by ≥1,000-fold to ≤10 and 20 min, respectively.

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The use of D2A21 for these studies does not represent an endorsement of this product.

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